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[54] **NOVEL MICROORGANISM AND USE THEREOF IN RIPENING CHEESE**

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[58] **Field of Search** 426/36, 38, 40, 582

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[57] **ABSTRACT**

The invention relates to *Lactobacillus helveticus* AGCl, a sample of which has been deposited on 29 September 1988 at The National Collections of Industrial And Marine Bacteria Limited under the accession number NCIB 40051, or a mutant or derivative thereof. This strain is useful as part of a starter culture addition to cheese milk, for accelerated ripening of cheddar style cheeses.

7 Claims, No Drawings

NOVEL MICROORGANISM AND USE THEREOF IN RIPENING CHEESE

This invention relates to a novel microorganism and its use in a method for the accelerated ripening of hard type cheeses of the Cheddar and related variant types, including low fat cheddar style cheese.

After initial manufacture, Cheddar cheese and Cheddar style varieties of cheese require a storage period of the order of four to six months at about 7° C., prior to sale . This storage period is necessary to allow the body of the cheese to acquire the typical characteristics of Cheddar cheese in terms of texture, consistency, and flavour. This extended storage period has evident disadvantages with respect to the financing costs of the cheese stocks involved, and with respect to marketing and production planning.

The development of typical Cheddar cheese, body, texture and flavour is the end result of complex physical and biochemical processes. These processes are influenced by a wide range of factors such as the composition and bacterial flora of the raw milk, the hygienic and manufacturing conditions used, the type of and condition of the starter bacteria used and the type of adventitious organisms present in the finished cheese. The composition of the cheese produced, the length of ripening period, and the temperature of storage during the ripening period are also important with respect to the development of a typical Cheddar cheese texture and flavour.

It is recognized that the body of the cheese is mainly modified from the initial 'curdy' texture and appearance of freshly produced cheddar cheese to that of a typical cheese as purchased by the consumer, by the proteolytic action of retained chymosin, by the proteolytic and peptidase enzymes, produced by the lactic acid starter bacteria and by adventitious organisms. These adventitious non-starter bacteria form the major flora of cheddar cheese after a relatively short period of ripening. The influence and interrelationships of these factors, in terms of cheese flavour and cheese texture, are as yet relatively undefined.

Manufacture of low fat cheeses presents problems in that flavour development is extremely slow and consequently such cheeses have a low consumer appeal.

A number of attempts to accelerate the flavour development of Cheddar and low fat Cheddar-style cheeses have been described.

EP-A-No. 0150743 describes a method for accelerating the aging process of Cheddar style cheese which is based on the use of preserved, partially disrupted preparation of the lactic acid bacterium *Lactobacillus caesi, Lactobacillus lactis, Lactobacillus olantarum* and a blend of pre-gastric lipases.

WO No. 82/03971 describes a method for the production of a low fat cheese product with enhanced flavour, using a culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and with a culture of *Lactobacillus caesi,* in addition to a normal cheese starter.

A number of methods to achieve accelerated ripening of Cheddar style cheeses, mainly based on enzyme additions are now commercially available or publicized (refs: 1,4,5,6).

However, one of the disadvantages of some of the systems is that the enzyme preparation has to be added to the cheese curd during the salt addition process and can give rise to enzyme distribution problems in the finished cheese, potentially resulting in flavour variations and physical defects such as cheese mottling. In the United Kingdom these systems have an additional disadvantage in that enzyme additions, other than coagulating enzymes, are not permitted under current cheese regulations, in traditional English type cheeses manufactured for direct sale to the consumer.

EP-A-No. 0150743 suffers from the disadvantage that cells of the lactic acid bacteria must be partially disrupted and then preserved before use. Such a process is technically difficult to operate and adds to the cost of cheese manufacture. WO No. 82/03971 suffers from the disadvantage that three cultures, in addition to a normal cheese starter, must be used.

The present invention relates to the surprising discovery that addition of a specific culture of *Lactobacillus helveticus* AGC1, characterized by its carbohydrate utilization pattern, and by its content of the nucleotides, guanine and cytosine (38.5%), to a typical manufacturing process for Cheddar and low fat Cheddar-style cheeses results in accelerated ripening.

The invention thus provides *Lactobacillus helveticus* AGC1, a sample of which has been deposited on 29 Sept. 1988 at The National Collections of Industrial and Marine Bacteria Ltd (NCIMB), P.O. Box 31, 135 Abbey Road, Aberdeen AB9 8DG, UK, under accession number NC1B 40051, or a mutant or derivative thereof.

L. helveticus AGC1 can be distinguished from other strains of *L. helveticus* by its characteristic carbohydrate utilization pattern (see Table 1). Thus, the invention provides a novel strain of *L. helveticus* which can be used to accelerate ripening of Cheddar style cheeses.

In practice, the additional culture is added at the normal point of starter culture addition to cheese milk, during the cheese making process. The rate of culture addition can be varied as required and is normally in the range of 0.01–1% and preferably 0.05% to 0.3%.

The addition of *L. helveticus* AGC1 in this manner may be regarded as the deliberate addition of a non-traditional (in this application) lactic acid starter bacterium, as a source of additional peptidases and proteolytic enzymes. This method produces the correct amount and type of breakdown in the body of the cheese, as determined by an expert cheese grader, and confirmed rheologically by means of measurements using an Instron universal tester instrument.

Novel aspects of our invention include the following:

1. A method for producing Cheddar cheese or Cheddar-like cheeses of composition in the range of 20–38% fat content and 30–45% moisture content made in a usual Cheddar cheese manufacturing type operation, with the specific addition of the identified strain of *Lactobacillus helveticus* AGC1 in addition to the usual cheese starter culture organisms. Such a method results in a cheese characterized by an accelerated ripening profile and in which unacceptable changes in texture do not occur.

2. A method for the production of a low fat Cheddar style cheese with a fat content in the range 8–20% and a moisture content in the range of 37–54% made with the specified strain of *Lactobacillus helveticus* AGC1, in addition to the usual cheese starter culture organisms. Such a method results in a cheese characterized by an accelerated ripening profile and in which unacceptable changes in texture do not occur.

L. helveticus AGC1 is a thermophilic strain and has an optimum temperature for growth that is higher than that of normal starter cultures. For that reason it is important that the scald temperature is maintained in

the range of 95°–108° F. (35°–42.2° C.). Within this temperature range growth of all the starter cultures is maintained in balance. At higher temperatures *L. helveticus* AGC1 will overrun traditional cultures resulting in a poor quality cheese.

The application of the invention is exemplified in the following Examples.

EXAMPLE 1

Typical Cheddar Cheese Manufacturing Schedule, with the addition of *Lactobacillus helveticus* AGC1

Raw Materials

A. Milk which should be clean fresh and free from off flavour, antibiotics or any other inhibitory substance and be of good bacteriological quality.
B. Starters—lactic cultures
   (i) Normal *S. lactis/S. cremoris* type. and
   (ii) *L. helveticus* AGC1
C. Rennet: Standard rennet, as necessary.
D. Anatto: Coloured variety only.
E. Salt: Standard cheese salt.

PROCESSING CONDITIONS

A Blend of normal starter cultures and the *L. helveticus* AGC1 culture, depending on the activity of the cultures and the type of acid profile required, is added at the rate of 0.5–2%. The L. *helveticus* AGC1 culture is added at the rate of 0.05–1%. Both cultures are added simultaneously at the beginning of the usual milk 'ripening' period, immediately prior to renneting. The scald temperature should be sufficiently high to promote growth of the *L. helveticus* AGC1 culture (i.e. in the range of 95°–108° F. (35°–42.2° C.). Temperatures outside this range may result in an excessive outgrowth of the *L. helveticus* ACG1 culture. No significant difference from control cheese should be noted in the rennet to mill time.

Salt addition must be targeted to give a salt level in the finished cheese of 1.6–1.8%. The uniformity of salt distribution is of prime importance. The culture will be inhibited by a high salt concentration, thereby nullifying the effect of accelerated ripening. The cheese is thereafter pressed and transferred to storage as per routine procedures.

Typical results are shown as compared to the control cheese, in Table 2, demonstrating the initial rapid textural change in cheese produced by the method of this application, as compared to the control sample. This process also results in the production of the appropriate level and type of proteinases and peptidases which, with the retained chysmosin, are responsible for the breakdown of the initial cheese body and the production of a typical body texture and flavour profile, or the precursor chemicals to produce characteristic Cheddar cheese flavour, body and texture.

One of the traditional flavour defects in Cheddar cheese is a "bitter" flavour, which is thought to be due to the production of hydrophobic peptides due to insufficient hydrolysis of the peptide chains. Our process appears to result in the rapid breakdown of peptides, in excess of that encountered in a control sample, with the production of greater amounts of single amino-acids, without the production of bitter 'off' flavours. An amino acid analysis of trichloroacetic acid (TCA) extracts of Control and Accelerated cheeses at various time intervals is given in Table 3.

We believe that the mode of action of the *L. helveticus* ACG1 culture is effectively the production of proteolytic and peptidase enzymes of the right quantity, type and character resulting in rapid breakdown of the protein structure to give the required texture, accompanied by extensive peptide hydrolysis, resulting in the production of a correct blend of amino acids, peptides, lipases, fatty acids, etc. which act as either the main flavour ingredient in cheese or the precursor for such flavour development. Our process produces a cheese which is ready for pre-packing or sale to consumer or customers in approximately 8 to 10 weeks, as shown in Table 2, as compared to sixteen to twenty weeks for cheese produced using standard techniques.

A typical comparison of the aging process for an accelerated Cheddar cheese, as compared to a control Cheddar cheese, is given in Table 2. In addition, surprisingly, we have noted that whereas flavour development continues at an accelerated rate, the texture changes in cheese produced by our method effectively reach a plateau for an indefinite period. In other words, once the initial texture changes are completed, evident, continuing unacceptable changes in texture do not occur, thus avoiding the production of an unacceptable product at any point in the life of the cheese.

EXAMPLE 2

Production of Low Fat Hard Type Cheese

To produce low fat cheese using *L. helveticus* ACG1 , particular attention needs to be given to certain key areas:

Standardization of the cheese milk and its heat treatment.
The blending and addition of the lactic cultures.
Setting and cutting of the curd.
Scalding, stirring and pitching the curd.
Curd treatment or cheddaring.
Salting.
Pressing.

Fresh raw milk needs to be standardized, by the addition of skim milk or by partial separation to a given fat percentage, or fat to protein ratio.

The fat levels will depend upon:

1. Fat required in the end product.
2. Fat lost in the whey, or conversely, fat retained in the cheese.
3. Protein retained in the cheese.

For most factory conditions, the fat in the cheese will probably be less than 17% fat, therefore a fat to protein ratio of the order of 1:2, is required.

The fat reduced milk is pasteurized at 161°–162° F. (71.7°–72.2° C.) and cooled to 88° F. (31.1° C.) for incubation.

A blend of cultures is added to the vat milk and this consists of the normal cultures of the day and *L. helveticus* ACG1. The amount of culture and the ratio between cultures is governed by:

1. Acid development required to give the correct Rennet to Mill time (normally 3 hours 20 minutes to 3 hours 40 minutes).
2. Rate of maturation required.

At ripening temperature of 88° F. (31.1° C.) significant acidity development from the *L. helveticus* AGC1 cultures is not expected, or encountered. Too high ripening temperatures will result in the *L. helveticus* AGC1 culture outgrowing the normal culture, and over maturation of the resulting cheese.

Starter addition mix needs to be in the order of 1–2% and the *L. helveticus* AGC1 being added at 5–15% of the total inoculum. The length of ripening influences the moisture retaining properties of the curd and at least 35–45 minutes is required. Acidity at the end of the period is normally in the range 0.16–0.19% lactic acid.

At the end of ripening, standard rennet is added at the rate of 40 oz per 1000 gallons (0.249 g/1) of cheese milk.

The cheese milk is allowed to settle for approximately 45 minutes, or until the curd is firm. The exact cutting operation will vary from plant to plant, but the aim is to cut the curd fairly large, so as to retain as much moisture as possible. The stirring of the curd should be as gentle as possible, but obviously sufficient to prevent the curd matting at the bottom of the vat.

Scald temperatures ranging from 94°–96° F. (34.4°–35.6° C.) are used. The curd is milled with a Cheddar chip mill at an acidity of 0.55% lactic acid.

Salt addition is targeted to give approximately 1.4–1.5% salt in the finished cheese. Under salting may result in rapid proteolysis with bitter off flavour, whereas over salting will give cheese of poor texture and sweeter flavours. The cheese is pressed following standard cheese pressing procedures.

Typical recipes for production of conventional low fat Cheddar cheeses and for production of low fat Cheddar cheeses using *L. helveticus* AGC1 are given in Table 4. A typical comparison of the aging process for an accelerated low fat Cheddar cheese, as compared to a conventional low fat Cheddar cheese, is given in Table 5. Cheese produced by our method develops a mature flavour by 7 weeks whereas conventional low fat cheese does not reach this level of flavour development unless stored for unrealistic periods (i.e. greater than 18 weeks). In addition, surprisingly, low fat cheeses produced by our method do not undergo any unacceptable changes in texture.

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	Fermentation Activity ** (3 = high, 2 = medium, 1 = low, 0 = negative)									
Carbon Source	AGC1	NCDO H3	NCDO H6	NCDO H13	NCDO H17	NCDO 28	NCDO 30	ATCC 39538	ATCC 39539	ATCC* 39542
Glucose	3	3	3	3	3	3	3	3	3	—
mannose	3	3	3	3	3	3	3	3	3	—
lactose	3	3	3	3	3	1	3	1	3	—
galactose	3	0	1	3	3	1	3	0	3	—
fructose	3	2	2	1	1	1	2	3	3	—
n-acetyl glucosamine	1	0	0	3	0	1	3	3	3	—
ribose	0	0	0	0	0	0	0	0	3	—
trehalose	0	0	0	3	0	0	0	3	3	—
maltose	0	0	0	0	0	0	0	1	0	—
sucrose	0	0	0	0	0	0	0	3	3	—
adonitol	0	0	0	0	0	0	0	0	1	—
mannitol	0	0	0	0	0	0	0	0	3	—
sorbitol	0	0	0	0	0	0	0	0	2	—
inulin	0	0	0	0	0	0	0	0	3	—
turanose	0	0	0	0	0	0	0	0	3	—
lyxose	0	0	0	0	0	0	0	0	3	—
sorbose	ALL TESTS NEGATIVE									
rhamnose										
methyl-glucoside										
amygdalin										
arbutin										
esculin										
cellobiose										
melibiose										
melezitose										
raffinose										
starch										
xylitol										
gentiobiose										
tagatose										
fucose										
arabitol										
gluconate										

-continued

Carbon Source	Fermentation Activity ** (3 = high, 2 = medium, 1 = low, 0 = negative)								
	AGC1	NCDO H3	NCDO H6	NCDO H13	NCDO H17	NCDO 28	NCDO 30	ATCC 39538	ATCC 39539	ATCC* 39542
keto-gluconate										

*Morphologically dissimilar from LBI
Not tested
**Based on readings 24h after incubation at 37° cC.
NCDO type cultures were all *L. helveticus* strains
ATCC 39538 was *L. lactis* strain
ATCC 39539 was *L. casei* strain
ATCC 39542 was *L. plantarum* strain

TABLE 2

TYPICAL AGEING PROFILE FOR ACCELERATED AS COMPARED TO CONVENTIONAL CHEDDAR CHEESE

AGE OF CHEESE	GRADING/COMMENTS VAT 9 (CONTROL)	VAT 10 (ACCELERATED)
3 weeks	Typical young curd Insipid	Good body and texture similar to 8–9 week cheese
7 weeks	Good body - texture Mild	Good body and texture, breaking down very nicely similar to 4–5 month Cheddar
9 weeks	Good body - texture Mild	Good body and texture, on par with 5–6 month Cheddar
10 weeks	Good body - texture Mild	Similar to a good 5–6 month Cheddar
12 weeks	Typical good 3 month old Cheddar	good body and texture equal to 6 month old
16 weeks	Good cheese 3–4 month age	Good body and texture close and smooth, equal to 6 months old
18 weeks	Good 4–5 month Cheddar	Equal to 6 month old
20 weeks	Good body and texture Reasonable flavour, equal to 5 month Cheddar	Good body and texture Close and smooth waxy, 7–8 month old
22 weeks	Good body and texture Reasonable flavour Equal to 5 month Cheddar	Good body and texture Close and smooth waxy 7–8 month old.

	GRADING AT 11 WEEKS OLD (NACPE POINTS)	
	VAT 9	VAT 10
Flavour	40	40
Body Texture	33	33
Colour	9	10
Finish	5	5
	87 points	88 points

TABLE 3

COMPARISON OF TCA SOLUBLE AMINO ACIDS IN CONTROL AND ACCELERATED CHEESES WITH TIME

TIME	Nano Moles/per ml of sample 20 days Control	20 days Accel.	84 days Control	89 days Accel.	172 days Control	172 days Accel.
Taurine	—*	—	—	—	—	—
Urea	—	—	—	—	—	—
Asp	40	45	44	190	79	300
Thre	9.1	17	14	69	29	140
Ser	12	21	11	98	18	120
Asn	—	—	—	—	—	—
Glu	87	140	130	610	240	980
Gln	—	—	—	—	—	—
Sarcosine	—	—	—	—	—	—
α-amino adipic	—	—	—	—	—	—
Pro	—	—	—	—	—	—
Gly	11	22	14	105	31	170
Ala	24	70	26	134	49	190
Citrulline	—	—	—	—	3.9	37
β-amino butyric	—	—	—	—	—	—
Val	20	41	39	220	80	360
Cystine	—	—	—	—	—	—
Met	2.4	2.0	11	46	23	85

TABLE 3-continued

COMPARISON OF TCA SOLUBLE AMINO ACIDS IN CONTROL AND ACCELERATED CHEESES WITH TIME

	Nano Moles/per ml of sample					
TIME	20 days Control	20 days Accel.	84 days Control	89 days Accel.	172 days Control	172 days Accel.
Cystathionine	—	—	—	—	—	—
Ileu	4.2	15	8.9	120	20	250
Leu	55	78	94	370	190	530
Tyr	7.7	13	9.2	70	11	110
Phe	30	41	52	170	100	250
β-amino butyric	—	—	—	—	—	—
acid	—	—	—	—	—	—
β-alanine	—	—	—	—	—	—
γ-amino butyric	—	—	—	—	—	—
acid	—	—	—	—	—	—
Ornithine	3.5	0.5	25	3.1	58	63
Lys	41	94	55	370	110	610
Tryp	—	—	—	—	—	—
His	7.0	15	8.4	83	14	170
1-Me-His	—	—	—	—	—	—
3-Me-His	—	—	—	—	—	—
Arg	23	46	18	170	22	120

*not determined

TABLE 4

LOW FAT CHEDDAR CHEESE RECIPES

	CONVENTIONAL	ACCELERATED
MILK	1100 gallons	1100 gallons
Fat	1.62% these will	1.62% these will
Protein	3.20% vary	3.20% vary
STARTER/S	Starter of the day but reduced by 10% of normal rate. Acidity: 1.58% LA	Starter of the day reduced by 15% of normal rate. Acidity: 1.58% LA 2 gallons of *L. helveticus* AGC1 culture. Acidity: 1.50% LA
RIPENING TEMP.	90° F.	90° F.
RIPENING TIME	30 mins	30 mins
RENNET	43 oz	43 oz
SETTING TIME	45 mins	45 mins
CUTTING SPEED	Speed 8 for 8 mins (large cut)	Speed 8 for 8 mins (large cut)
SCALD TEMP.	92° F. (Acidity: 0.12% LA) Scald up in 20 mins (Acidity: 0.125% LA)	96° F. (Acidity 0.12% LA) Scald up in 20 mins (Acidity: 0.125% LA)
PITCHED	10 minutes after scald up	10 minutes after scald up
WHEY OFF	15 minutes (Acidity: 0.15% LA)	15 minutes (Acidity: 0.16% LA)
ACID DEVELOPMENT (Time from Renneting)	2 hrs 0.18% LA 2.5 hrs 0.27% LA 3 hrs 0.35% LA 3.5 hrs 0.49% LA 3 hrs 50 mins 0.57% LA	2 hrs 10 mins 0.21% LA 2 hrs 40 mins 0.27% LA 3 hrs 20 mins 0.45% LA 3 hrs 50 mins 0.55% LA
MILLED	@ 0.57% LA	@ 0.55% LA

TABLE 5

TYPICAL AGEING PROFILE FOR ACCELERATED AS COMPARED TO CONVENTIONAL LOW FAT CHEDDAR CHEESE

	GRADING/COMMENTS	
AGE OF CHEESE	CONVENTIONAL (NORMAL STARTER)	ACCCELERATED (NORMAL STARTER + *L. HELVETICUS* AGC1)
3 weeks	Good body and texture Mild flavour	Good body and texture Clean, good flavour, more flavour than conventional
4 weeks	Good body and texture Clean, mild flavour	Good body and texture Good, clean flavour
6 weeks	Good body and texture Clean, mild flavour	Good body and texture Good, clean, well developed flavour

TABLE 5-continued

TYPICAL AGEING PROFILE FOR ACCELERATED AS COMPARED TO CONVENTIONAL LOW FAT CHEDDAR CHEESE

	GRADING/COMMENTS	
AGE OF CHEESE	CONVENTIONAL (NORMAL STARTER)	ACCCELERATED (NORMAL STARTER + *L. HELVETICUS* AGC1)
7 weeks	Good body and texture Clean, mild flavour	Good body and texture Good, clean, mature flavour
11 weeks	Good body and texture Clean flavour	Good body and texture Good, clean mature flavour
18 weeks	Good body and texture Clean and slightly	Good body and texture Good mature flavour

TABLE 5-continued

TYPICAL AGEING PROFILE FOR ACCELERATED AS COMPARED TO CONVENTIONAL LOW FAT CHEDDAR CHEESE		
	GRADING/COMMENTS	
AGE OF CHEESE	CONVENTIONAL (NORMAL STARTER)	ACCCELERATED (NORMAL STARTER + *L. HELVETICUS* AGC1)
	sweet	

I claim:

1. A method of making cheddar-style cheeses which comprises:

a. adding to milk a mixture of starter cultures which includes Lactobacillus helveticus AGC1 (NCIB 40051);

b. permitting the milk to ripen;

c. adding rennet to the ripened milk;

d. permitting the resulting milk to set and form a curd;

e. cutting the curd, stirring and heating to scald temperature;

f. separating the curd from the whey;

g. cheddaring the curd; and

h. recovering the cheeses.

2. A method according to claim **1** wherein the curd from step *g* is milled, salted, pressed and allowed to mature.

3. A method according to claim **1**, in which the cheese has a fat content of 20 to 38% and a moisture content of 30 to 45%.

4. A method according to claim **1**, in which the cheese has a fat content of 8 to 20% and a moisture content of 37 to 54%.

5. A method according to claim **1**, in which *Lactobacillus helveticus* AGC1 is added to a proportion of 0.05 to 0.3%, based on the total weight of the starting milk.

6. A method according to claim **1**, in which the scald temperature is in the range of 94° to 108° F.

7. A method according to claim **1** wherein the starter cultures are added to the milk in a proportion of 0.0l to 1%.

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